Lysostaphin

Week 12

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 PCR of pSB1C3-T7-Lys and pSB1C3-T7-Lys-LT

Responsible

Reskandi Rudjito

Protocols used

- PCR
- Gel electrophoresis

Experimental Set Up

Two PCR reactions were performed on plasmids consisting of pSB1C3-T7-Lys and pSB1C3-T7-Lys-LT. The PCR was done with VF2 and VR primers. The purpose of this PCR was to confirm that the sample that had been named 'pSB1C3-T7-Lys-LT' was infact pSB1C3-T7-Lys. This is confirmed if the two PCR products show bands of the same size.
Table 1 shows the composition used for the PCR and Table 2 shows the PCR conditions used in the thermocycler. The PCR reaction used for each sample was 25 μl.

Table 1: PCR Reaction

Master Mix	Component	Volume [μl]
1	PCR Grade Nucleotide mix	2
	R_Primer (VR)	10
	F_primer (VF2)	10
	Template DNA	1
	Sterile water	27
	Total Volume	50
2	PCR Reaction buffer 10 x	10
	Taq DNA Polymerase	2.5
	Sterile water	37.5
	Total Volume	50

Table 2: PCR condition using Taq polymerase

Step	Cycles	Temperature {[°C]}	Time
Initial Denaturation	1	94	2 min
Denaturation	30	94	30 secs
Annealing		52	1 min
Extension		72	1 min
Final Extension	1	72	7 min
Hold	indefinitely	4	-

Sample Calculation

No further calculations were done.

Results and Conclusions

The result of the PCR with the verification primers show bands of the same size that being around 1000 bp.

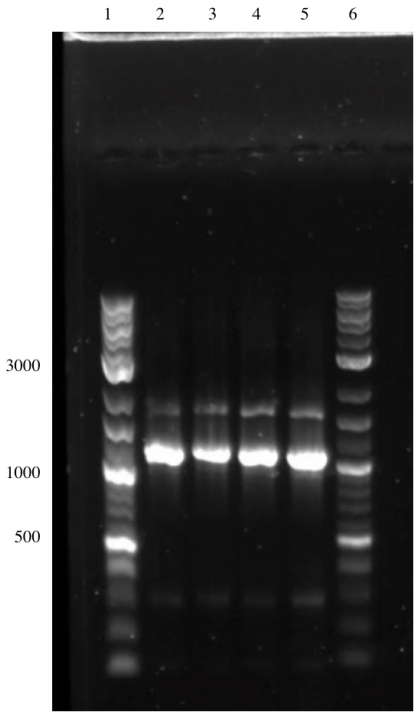

Figure 1: PCR of pSB1C3-T7-Lys and pSB1C3-T7-Lys-LT. (1) Ladder (2) Sample pSB1C3-T7-Lys1 (3) Sample pSB1C3-T7-Lys2 (4) Sample pSB1C3-T7-Lys-LT1 (5) pSB1C3-T7-Lys-LT2 (6) Ladder

Discussion and Troubleshooting

The amplified fragments of pSB1C3-T7-Lys and pSB1C3-T7-Lys-LT should be 1093 bp and 1147 bp in size, respectively. Based on the ladder from Figure 1, all four bands exhibit a size slightly larger than 1000 bp. However, since the migration of the DNA was not very smooth we focused on the position of the bands when compared to each other. The four bands are positioned very similarly to each other, and this confirms the fact that the linker tag (LT) was not successfully assembled.

2 Revival of *E. coli* TOP 10 cells glycerol stocks containing pSB1C3-T7-Lys

Responsible

Reskandi Rudjito

Protocols used

- Glycerol stock

Experimental Set Up

Glycerol stocks of *E. coli* TOP 10 cells containing pSB1C3-T7-Lys were cultured in LB medium containing chloramphenicol. The plasmids within the cells were then extracted using Mini prep.

Sample Calculation

No calculations were done.

Results and Conclusions

The result of the Mini prep extraction is shown in the table below. We aimed at getting approximately 250 ng/μl in order to achieve high enough concentrations for the plasmids to be sent in as biobricks.

Table 3: Plasmid concentrations after Mini Prep

Sample	Concentration (ng/μl)
Lys 1	180
Lys 2	291